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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

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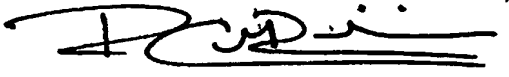

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

USE OF FOLLICLE STIMULATING HORMONE FOR REDUCTION OF SPERMATOZOA CHROMOSOMAL ABERRATION IN MALES

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USE OF FOLLICLE STIMULATING HORMONE FOR REDUCTION OF SPERMATOZOA CHROMOSOMAL ABERRATION IN MALES

Field of the invention

This invention relates to the use of a substance selected from Follicle Stimulating Hormone (FSH) and FSH variants for reducing gamete chromosomal alterations in the male. More specifically, the invention provides the use of a substance selected from FSH and FSH variants in men suffering from spermatozoa aneuploidy, notably diploidy. The invention further comprises the use of pharmaceutical formulations containing Follicle Stimulating Hormone (FSH) and FSH variants for the preparation of a pharmaceutical composition for the treatment and/or prevention of gamete chromosomal alterations in the male.

Background of the invention

A considerable percentage of infertile men have an abnormal karyotype and, therefore, these subjects produce gametes with chromosomal alterations. Furthermore, having a normal karyotype does not exclude the possibility that spermatozoa with chromosomal alterations are present, since errors in chromosomal segregation can occur during the mitotic and/or meiotic division in spermatogenesis. Based on the first data available in the literature (Vegetti et al., 2000), it has been suggested that approximately 0.3–1.08% of the spermatozoa of normal men have numerical chromosomal aberrations and that this percentage becomes higher when examining the spermatozoa of men with oligospermia (0.7–9.44%) and teratospermia (1.3–3.9%). These alterations appear to be related to the sex chromosomes and autosomes, particularly chromosomes 1, 18, and 21.

Numerical chromosomal aberration characterized by extra or missing chromosomes is called "aneuploidy".

Different types of aneuploidies are known and they are designated according to the kind of chromosomal numerical aberration: for example, the presence of one additional chromosome compared to the normal number in diploid cells is known as trisomy (2n +1); the absence of one chromosome in a homologous pair in diploid cells is known as monosomy (2n-1), while the loss of an entire chromosomal pair in diploid cells is known as nullisomy (2n-2), wherein n is the number of types of chromosomes.

By analogy, in the case of haploid cells, such as gametes, the term "aneuploid" characterizes gametes having a chromosomal numerical aberration, such as for example having an extra chromosome or missing one chromosome. These "aneuploid" gametes will form, when fused with a normal gamete, a zygote having an abnormal number of chromosomes (aneuploidy). Most of the time, the aneuploid zygotes die during the time between conception and birth. However, in some cases, the zygote becomes an offspring affected by "aneuploidy" and the consequences on the carrier of this chromosomal numerical aberration depends on the chromosomes which are involved.

The development of gametic aneuploidy of autosomes and sex chromosomes results from errors, such as failed separation of brother chromatids that occur during mitosis of spermatogonia and during the first and second meiotic division or such as failed separation of homologous chromosomes in meiosis. When the chromosomes fail to separate during the first meiotic phase, all the produced spermatozoa will have an aberrant number of chromosomes (only 2n:diploids or 0n). If the chromosomes fail to separate during the second meiotic phase, only 2 haploid cells out of the 4 coming from the same spermatocyte will be affected by chromosomal numerical aberration.

Therefore production of gamete aneuploidies constitutes a serious genetic risk factor as they induce aneuploidy in the offsprings which causes a lot of abnormalities in the foetus and in the viable infants.

For example, aneuploidy can cause infertility, miscarriages, perinatal mortality, congenital malformations, mental retardation, and abnormal behavior (Hook, 1985; Hecht et al., 1987), increased sensitivity to infectious diseases, high propensity of leukeamia or early development of Alzheimer disease in the offspring.

Aneuploidies in autosomes such as monosomies are always lethal for the foetus whereas trisomies are non lethal only when they involove chromosomes 13, 18 and 21 but dramatically handicapping for the offspring.

Examples of common sex chromosome aneuploidy are found in patients with Klinefelter Syndrome (47, XXY) which is the most common form of aneuploidy in men and in patients (47, XYY) which is another form of aneuploidy discovered by Sandberg and co-wokers in 1961.

The presence of spermatozoa chromosomal abnormalities (both numerical and structural) has been noted in a considerable percentage of infertile patients (Bourrouillou et al., 1985) and it is widely accepted that using assisted fertilization techniques allows these patients to procreate, increasing the risk of having children with chromosomal alterations.

Aneuploid gametes also develop in subjects with oligoasthenoteratozoospermia (OAT) with normal chromosomal sets since noxious testicular pathogens can disrupt the delicate process of chromosomal segregation during spermatogenesis. For this reason, evaluating the frequency of aneuploidy in spermatozoa is becoming a fundamental stage in the diagnostic course of infertile patients, particularly if they intend to undergo assisted fertilization techniques.

Published data indicate that patients with OAT have an increased percentage of spermatozoa with hereditary chromosomal aneuploidy. This increased frequency of aneuploidy reduces the fertilizing capability of spermatozoa during assisted fertilization techniques (Storeng et al., 1998). Considering the fact that spermatozoa separation methods used during fertilization *in vitro* and embryo transfer (FIVET) do not modify the percentage of aneuploid sperm, patients with OAT are at great risk of producing children with chromosomal aneuploidy (Pfeffer et al., 1999).
Research in this area has become more clinically relevant in the past few years with the development of intra-cytoplasmic sperm injection (ICSI). ICSI has been shown to be extremely useful for the treatment of infertility, howver transmission of cytogenic defects to offspring is a major concern with this fertilization technique and has been observed (Ushijima et al., 2000).
For this reason, spermatic aneuploidy should be assessed in all patients with OAT, particularly in those who intend to undergo assisted fertilization techniques.

It would be desirable to develop methods for reducing the rate of aneuploidy such as diploidy in spermatozoa, especially in men who intend to undergo Assisted Reproduction Techniques.

Follicle-stimulating hormone (FSH) is known for its role in the initial development of Sertoli cells and in their stimulation for controlling spermatogonia. It is suggested by experimental studies that FSH is present in Sertoli cells and in round germinal cells that both express the FSH receptor (Baccetti et al.,1998).

Furthermore, exogenous FSH therapies induced improvements in the structural characteristics of spermatozoa visualized through electron microscopy (Baccetti et al., 1997).

FSH is used to induce spermatogenesis in men suffering from oligospermia. A regimen using 150 IU FSH, 3 times weekly in combination with 2'500 IU hCG (human Chorionic Gonadotrophin) twice weekly has been successful in achieving an improvement in sperm count in men suffering from hypogonadotrophic hypogonadism (Burgues et al., 1997). High dose FSH (150 IU) has been used to treat iodopathic oligospermia (Iacono et al., 1996).

Summary of the invention

It is an object of the invention to provide a method for the reduction and/or prevention of gamete chromosomal alterations in a male, notably spermatozoa aneuploidy, including diploidy, preferably in men intending to undergo assisted fertilization techniques, whereby a pharmaceutically active amount of FSH or FSH variant is administered to the male in need thereof.

In a first aspect, the invention provides a use of a substance selected from Follicle Stimulating Hormone (FSH) and FSH variants for the preparation of a pharmaceutical composition for the treatment and/or the reduction of gamete chromosomal alterations in a male, notably spermatozoa aneuploidy, including diploidy.

In a second aspect, the invention provides a method for treating and/or preventing a disease or disorder associated with gamete chromosomal alterations in a male subject, notably spermatozoa aneuploidy, including diploidy, comprising administering to a patient in need thereof an effective amount of a substance selected from FSH and an FSH variant, wherein the male is a human male.

In a third aspect, the invention provides a method for reducing gamete numerical chromosomal alterations in a male subject, notably spermatozoa aneuploidy, including diploidy, comprising administering an effective dose of a substance selected from FSH and an FSH variant to the patient.

In a fourth aspect, the invention provides a method for presenting the occurrence of chromosomal aberrations in the offspring of a male subject, comprising administering an effective dose of a substance selected from FSH and an FSH variant to the male subject prior to conception.

Detailed description of the invention

The following paragraphs provide definitions of various terms, and are intended to apply uniformly throughout the specification and claims unless an otherwise expressly set out definition provides a different definition.

"Oligozoospermia" is diagnosed in men when sperm concentration $<20 \times 10^6$/ml.

"Asthenozoospermia" is diagnosed in men when fewer than 50% spermatozoa have forward progression or fewer than 25% spermatozoa have rapid linear progression.

"Teratozoospermia" is diagnosed in men when fewer than 30% spermatozoa have normal morphology.

"Oligoasthenoteratozoospermia" is diagnosed when of all three previously cited variables in sperm quality are disturbed, according to WHO criteria, i.e. when patients display the following parameters: density $<20 \times 10^6$ spermatozoa/mL, motility of grade 3 <25% and/or motility of grade 2 plus 3 <50%, and normal morphology in less than 30% of spermatozoa.

Exemplary diseases where chromosomal aberration are involved include Down's syndrome, Klinefelter's syndrome (XXY), Turner's syndrome (XO), Triplo-X syndrome, Tetra-X syndrome, Penta-X syndrome, XYY syndrome, monosomies or polysomies of any chromosome including 7, 10, 11, 13, 18, 21, X, and Y, sex chromosome aneuploidy in men with chromosomal mosaicism (XY/XXY), multiple X chromosomes (e.g. XXXXY; XXXYY).

The term "administer" or "administering" means to introduce a formulation of the present invention into the body of a patient in need thereof to treat a disease or condition.

The term "chromosomal aberration" is used for chromosomal abnormalities and includes chromosomal numerical aberrations such as polypleuidy and aneuploidy which includes

the presence of at least an extra chromosome or the absence of one chromosome. Aneuploidy in haploid cells such as gametes includes diploidy.

"Aneuploidy" and "polyploidy" are conditions in which a cell has a number of chromosomes different from the usual haploid number ("n") or diploid number ("2n"). In humans, the normal haploid number of chromosomes is 23, and the diploid number is 46. A normal somatic cell should have a diploid chromosome content of 23 pairs, or 46 chromosomes in total.

An aneuploid somatic cell has a number of chromosomes that is other than twice the normal haploid number (2n). For example, an aneuploid somatic cell can be a cell having a trisomy, i.e., a cell having three copies of one chromosome, or a monosomy, i.e., a cell having a single copy of one chromosome. Aneuploidy can result from chromosomal non-disjunction during mitosis. A polyploid cell is a cell having a number of chromosomes that is some multiple of the normal haploid number greater than the usual diploid number, i.e., 2n. For example, a polyploid cell can be triploid (n=69) or tetraploid (n=92) cell.

In a haploid cell, such as a gamete, the natural state is haploid, meaning n chromosomes. Aneuploidy means that the cell has a chromosome number that is other than n. For example, one chromosome may be missing (n-1), or an extra chromosome may be added (n+1), or a cell may be diploid (2n).

The term "patient" means a male mammal that is treated for a disease or condition. Patients are of, but not limited to, the following origin, human, ovine, porcine, equine, bovine, rabbit and the like.

The term "Assisted Reproduction Technology" includes IVF (In Vitro Fertilization), ICSI (intra-cytoplasmic sperm injection).

By "effective amount", is meant an amount of FSH sufficient to reduce gamete chromosomal alterations in a male subject, especially to reduce the rate of aneuploidy, including diploidy in a male patient. The amount of FSH can be routinely determined by those of skill in the art. The amount of the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual

compound administered, the age, weight, and response of the individual patient, the patient's endogenous FSH levels, and the like. The "effective amount" can alternatively be achieved by the duration of the treatment. Typically, the duration of the treatment includes treatments of at least one month, three month, six months or one year.

The rate of diploidy or aneuploidy can be determined through the evaluation of numerical chromosomal abnormalities in ejaculated spermatozoa for example by fluorescence *in situ* hybridisation (FISH), especially multicolour FISH. FISH involves hybridisation of specific DNA probes labelled with fluorochromes to complementary DNA sequences on target chromosomes, followed by detection of the bound probes under a fluorescence microscope as described in Shi et al., 2001. To perform FISH analysis on semen samples from donors with extremely low quantities of sperm, a microwave decondensation/codenaturation technique has been developed to increase analyzable sperm numbers for use in fluorescence in situ hybridization (FISH) (Rademaker et al., 2001). Example of a FISH protocol for determining the rate of aneuploidy or diploidy is detailed in Example n° 1.

The expression "FSH variant" is meant to encompass those molecules differing in amino acid sequence, glycosylation pattern or in inter-subunit linkage from human FSH but exhibiting FSH-activity. Examples include CTP-FSH, a long-acting modified recombinant FSH, consisting of the wild type α-subunit and a hybrid β-subunit in which the carboxy terminal peptide of hCG has been fused to the C-terminal of the β-subunit of FSH, as described in (La Polt et al., 1992 or Klein et al., 2003). Also included is single chain CTP-FSH, a single chain molecule, consisting of the following sequences (from N-terminal to C-terminal):

βFSH	βhCG-CTP(113-145)	αFSH

wherein βFSH signifies the β-subunit of FSH, βhCG CTP (113-145) signifies the carboxy terminal peptide of hCG and αFSH signifies the α-subunit of FSH, as described by Klein et al., 2003. Other examples of FSH variants include FSH molecules having additional glycosylation sites incorporated in the α- and/or β-subunit, as disclosed in WO 01/58493, particularly as disclosed in claims 10 and 11 and FSH molecules with inter-subunit S-S bonds, as disclosed in WO 98/58957.

The FSH variants referred to herein also include the carboxy terminal deletions of the beta subunit that are shorter than the full length mature protein.

FSH or FSH variants can be produced by any suitable method, such as recombinantly, by isolation or purification from natural sources as may be the case, or by chemical synthesis, or any combination thereof.

The use of the term "recombinant" refers to preparations of FSH or FSH variants that are produced through the use of recombinant DNA technology (see for example WO 85/01958).

The FSH used in accordance with the present invention may be produced not only by recombinant means, including from mammalian cells, but also may be purified from other biological sources, such as from urinary sources, e.g. urinary FSH or uFSH. Acceptable methodologies include those described in Hakola, 1997; Keene et al., 1989; Cerpa-Poljak et al., 1993; Dias et al. 1994; Flack et al., 1994; and Valove, et al., 1994, U.S. Patent 3,119,740 and US Patent no. 5,767,067.

The expression "Pharmaceutically acceptable" is meant to encompass any carrier or salt which does not substantially interfere with the effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which is administered.

The compounds of the present invention while effective in the form of the free form may be formulated and administered in the form of the therapeutically or pharmaceutically acceptable acid addition salts for purposes of stability, convenience of crystallization, increased solubility and the like, These acid addition salts include inorganic acid salts such as hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, perchloric acid salts and the like; and organic acid salts such as acetic, trifluoroacetic, propionic, oxalic, hydroxyacetic, methoxyacetic, 2-hydroxypropanoic, 2-oxopropanoic, propanedioic, 2-hydroxy-butanedioic, benzoic, 2-hydroxybenzoic, 4-amino-hydroxybenzoic, 3-phenyl-propenoic, alpha-hydroxybenzeneacetic, methanesulfonic, ethanesulfonic, benzenesulfonic, toluenesulfonic, cyclohexanesulfamic, succinic, tartaric, citric, maleic, fumaric acid salts and the like. The preferred acid addition salts are chloride, oxalate and citrate.

These acid addition salts can be prepared by conventional methods, such as by treatment of the free base of the inventive compound with the appropriate acid. The compounds of the present invention, prepared in the free form, can be combined with a

pharmaceutically acceptable carrier to provide a pharmaceutical composition. Suitable carriers for the free bases include propylene glycol-alcohol-water, isotonic water, sterile water for injection (USP), emulphor TM alcohol-water, cremophor-ELTM or other suitable carriers known to those skilled in the art, The compounds of the present invention, prepared in the pharmaceutically acceptable acid addition salt form, can also be combined with a pharmaceutically acceptable carrier to provide a pharmaceutical composition. Suitable carriers for the acid addition salts include isotonic water, sterile water for injection (USP), alone or in combination with other solubilizing agents such as ethanol, propylene glycol, or other conventional solubilizing agents known to those skilled in the art.

Of course, the type of carrier will vary depending upon the mode of administration desired for the pharmaceutical composition as is conventional in the art.

A preferred carrier is an isotonic aqueous solution of the inventive compound. The compounds of the present invention can be administered to mammals, e.g., animals or humans, in amounts effective to provide the desired therapeutic effect. Since the activity of the compounds and the degree of the desired therapeutic effect vary, the dosage level of the compound employed will also vary. The actual dosage administered will also be determined by such generally recognized factors as the body weight of the patient and the individual hypersensitiveness of the particular patient. Thus, the unit dosage for a particular patient (man) can be as low as about 0.00005 mg/kg, which the practitioner may titrate to the desired effect. The FSH or FSH variant can be administered parenterally, in the form of sterile solutions or suspensions, such as intravenously, intramuscularly or subcutaneously in the carriers previously described. The compounds may also be administered orally, in the form of pills, tablets, capsules, troches, and the like, as well as sublingually, rectally, or trans-cutaneously with a suitable pharmaceutically acceptable carrier for that particular mode of administration as is conventional in the art.

For parental therapeutic administration, the compounds of the present invention may be incorporated into a sterile solution or suspension. - These preparations should preferably contain at least at or about 0.001% of FSH or FSH variant by weight. The amount may be varied to at or about 50% of FSH or FSH variant by weight of the parental composition. The exact amount FSH or FSH variant present in such compositions is such that a suitable dosage level will be obtained. Preferred compositions and preparations

according to the present invention are prepared so that a paranteral dosage unit contains from between about 0.5 milligrams to about 100 milligrams of the inventive compound. The sterile solutions or suspensions may also include the following adjuvants: a sterile diluent, such as water for injection, saline solution, fixed oils, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antibacterial agents,, such as benzyl alcohol or methyl paraben; antioxidants, such as ascorbic acid or sodium metabisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates or phosphates; and agents for the adjustment of tonicity,, such as sodium chloride or dextrose, The parental preparations may be enclosed in ampules, disposable syringes,, or multiple dose vials made of glass or plastic. The compounds of the present invention can also be administered orally, For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like. These preparations should contain at least about 4% of the inventive compound, by weight, but this amount may be varied depending upon the particular dosage form from between about 4% to about 70% of the inventive compound, by weight of the oral composition. The exact amount of the compound present in the composition is such that a suitable dosage will be obtained. Preferred compositions and preparations according to the present invention are prepared so that an oral dosage unit form contains from between about 5 to about 300 milligrams of the inventive compound. The tablets, pills, capsules, troches and the like may also contain the following adjuvants: a binder, such as microcrystalline cellulose, gum agacanth or gelatine; an excipient, such as starch or lactose; a disintegrating agent, such as alginic acid, Primogel, corn starch and the like; a lubricating agent,, such as magnesium stearate or Sterotex; a gliding agent, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and a flavoring agent, such as peppermint, methyl salicylate or orange flavoring, When the dosage form is a capsule, it may additionally contain a liquid carrier such as a fatty oil, other dosage unit forms may contain other materials which modify the physical form of the dosage unit, such as enteric coatings. Thus tablets or pills may be coated with sugar, shellac, or other enteric coating agents. A syrup may contain, in addition to the above adjuvants, sucrose as a sweetening agent, preservatives, dyes, coloring agents and flavoring agents, It is especially advantageous to formulate the pharmaceutical compositions in dosage unit forms for ease of administration and uniformity of dosage. The term dosage unit forms as used herein refers to physically discrete units suitable for use as a unitary dosage, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the

pharmaceutical carrier.

Examples of such dosage unit forms are tablets (including scored or coated tablets), capsules, pills, powder packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

FSH is currently formulated for intramuscular (IM) or subcutaneous (SC) injection. It is supplied in a lyophilised (solid) form in vials or ampoules of 75 IU/vial and 150 IU/vial with a shelf life of one and a half to two years when stored at 2-25°C. A solution for injection is formed by reconstituting the lyophilised product with water for injection (WFI). Depending on the patient's response, up to three cycles of treatment with increasing doses of FSH can be used. With lyophilised formulations, the patient is required to reconstitute a new vial of lyophilised material with diluent and administer it immediately after reconstitution on a daily basis: for example: [Package insert N1700101A, published in February 1996, for Fertinex™ (urofollitropin for injection, purified) for subcutaneous injection, by Serono Laboratories, Inc., Randolph, MA] and Puregon® (Follitropin beta for injection) for subcutaneous injection, by N.V.Organon, BH OSS, Netherlands).

FSH has also been formulated in both single-dose and multi-dose liquid formats, in vials, or ampoules. Single dose formats must remain stable and potent in storage prior to use. Multi-dose formats must not only remain stable and potent in storage prior to use, but must also remain stable, potent and relatively free of bacteria over the multiple use regimen administration period, after the seal of the ampoule has been compromised. For this reason, multi-dose formats often contain a bacteriostatic agent.

Examples of formulations for FSH are listed below.

EP 0 618 808 (Applied Research Systems ARS Holding N.V.) discloses a pharmaceutical composition comprising a solid intimate mixture of gonadotrophin and a stabilising amount of sucrose alone or in combination with glycine.

EP 0 448 146 (AKZO N.V.) discloses a stabilized gonadotrophin containing lyophilisate comprising one part by weight of a gonadotrophin; and 200 to 10,000 parts by weight of a dicarboxylic acid salt stabilizer associated with the gonadotrophin.

EP 0 853 945 (Akzo Nobel N.V.) discloses a liquid gonadotrophin-containing formulation characterised in that the formulation comprises a gonadotrophin and stabilising amounts of a polycarboxylic acid or a salt thereof and of a thioether compound.

WO 00/04913 (Eli Lilly and Co.) discloses a formulation comprising FSH or an FSH variant, containing an alpha and beta subunit, and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent.

Alternatively, the levels of FSH may be increased in a male suffering from gamete chromosomal alterations, by raising endogenous FSH levels through the administration of GnRH agonists, preferably by pulsatile administration as disclosed by Hetzel et al., 1985. GnRH agonists include, for example, Buserelin, Goserelin, Leuprorelin, Triptorelin and Nafarelin.

The levels of FSH may be increased in a male suffering from gamete chromosomal alterations, by raising endogenous FSH levels through the administration of FSH agonists, for example the FSH agonists described in WO 0209706 and WO 0008015.

In one embodiment, the invention provides a use a substance selected from Follicle Stimulating Hormone (FSH) and FSH variants for the preparation of a pharmaceutical composition for the treatment and/or the reduction of gamete chromosomal alterations in a male, notably numerical aberrations in spermatozoa such as gamete aneuploidy, including diploidy.

In another embodiment, the invention provides a method for treating and/or preventing a disease or disorder associated with gamete chromosomal alterations in a male, notably numerical aberrations in spermatozoa such as gamete aneuploidy, including diploidy, comprising administering to a male subject in need thereof an effective amount of a substance selected from FSH and FSH variants, wherein the subject can be human or animal.

In another embodiment, the invention provides a method for reducing gamete chromosomal alterations in a male, notably numerical aberrations in spermatozoa such as gamete aneuploidy, including diploidy, comprising administering an effective dose of a substance selected from FSH and FSH variants to the patient.

In another embodiment, the invention provides a method for preventing the occurrence of chromosomal aberrations in the offspring of a male subject, comprising administering an effective dose of a substance selected from FSH and FSH variants to the male, prior to conception.

In another embodiment, the invention provides a method of in vitro fertilization, including ISCI, comprising the step of treating the donor of spermatozoa, prior collection of spermatozoa, with an amount of FSH or FSH variant sufficient to prevent or reduce chromosomal aberration in the spermatozoa.

In a preferred embodiment of the invention, the male is human.

In another preferred embodiment of the invention, the substance is FSH.

In another preferred embodiment of the invention, the substance is rFSH.

In another preferred embodiment of the invention, the male presents a numerical chromosomal aberration in spermatozoa.

In another preferred embodiment of the invention, the numerical chromosomal aberration in spermatozoa is a diploidy.

In another preferred embodiment of the invention, the male presents an aneuploidy in spermatozoa at least or about 1%.

In another preferred embodiment of the invention, the male presents a diploidy in spermatozoa of at least or about 0.5%.

A preferred group of patients is represented by males presenting oligospermia or teratospermia.

Another preferred group of patient is represented by males presenting a diploidy of at least or about 0.8%.

In another preferred embodiment of the invention, the administration of the substance is performed on alternate days.

In another preferred embodiment of the invention, the substance is administered at or about 75 to 300 IU/dose, preferably at 150 IU/dose.

In another preferred embodiment of the invention, the patient will undergo Assisted Reproduction Technology such as IVF (In vitro fertilization), ISCI (Intracytoplasmic Sperm Injection).

In another preferred embodiment of the invention, the treatment of the patient substance selected from FSH and FSH variant to the male is performed during at least a time selected from one week, one month, three months, six months and one year, preferably at least three months before undergoing Assisted Reproduction Technology.

In one more preferred embodiment, the invention provides a use of rFSH for the preparation of a pharmaceutical composition for the treatment and/or the reduction of spermatozoa aneuploidy in a patient.

In another more preferred embodiment, the invention provides for reducing spermatozoa aneuploidy in a patient, comprising administering an effective dose of a substance selected from rFSH to the patient.

The invention will now be described by means of the following Examples, which should not be construed as in any way limiting the present invention. The Examples will refer to the Figures specified here below.

Abbreviations:

CEP (Chromosome Enumeration Probes); DHEA (Dehydroepiandrosterone); DTT (dithiothreitol, Biorad); EDTA (ethylenediaminetetraacetic acid); FIVET (Fertilization In Vitro and Embryo Transfer); FISH (Fluorescent IN SITU Hybridization); FSH (Follicle Stimulating Hormone); GnRH (Gonadotropin Releasing Hormone); hCG (human Chorionic Gonadotropin); ICSI (Intracytoplasmic Sperm Injection); IM (intramuscular);

IVF (In Vitro Fertilization); IU (International Unit); LIS (3,5 di-iodosalicylic acid lithium salt); OAT (oligoasthenoteratozoospermia); rFSH (Recombinant FSH); RT (room temperature); SC (subcutaneous); Thyroid Stimulating Hormone (TSH); uFSH (urinary FSH); USP (United States Pharmacopeia); WFI (Water For Injection).

EXAMPLES

The invention will be illustrated by means of the following examples which are not to be construed as limiting the scope of the invention.

EXAMPLE 1: Effe ct of Gonal F on aneuploidy in spermatozoa of human infertile males.

a) Selection of infertile patients

Their infertility is determined after an andrological visit comprising spermiogram record, hormonal measurement (Androstenedione (A), total testosterone (Ttot), DHEAs, Prolactine (PRL), TSH, free tyroxine (FT4)) and microdeletion of chromosome Y.

Normal values are the following: A: 1200-5000 ng/ml), T tot: 1500-11400 pg/ml, Prl: 2-15 ng/ml; FT4: 50-120 ng/ml; FSH: 0.7 – 10 mIU/ml.

Blood samples are obtained between 9-11 a.m. Measurements are performed by commercial RIA or ELISA kits.

b) Aneuploidy/diploidy rates in sex chromosomes determination by FISH analysis before treatment

Fresh sperm samples were washed with 150 mM NaCl and 10 mM Tris-HCl (pH 8), smeared on glass slides and dried in air. They were then fixed in 3:1 methanol-acetic acid for 10 min. The slides were dehydrated in 70%, 80% and 100% cold ethanol and air dried. Samples were swollen treated with 0.01 M DTT (dithiothreitol, Biorad) in 0.1M Tris-HCl (pH 8) and then 20 mM LIS (3,5 di-iodosalicylic acid lithium salt, Sigma) in the same buffer, checking sperm head swelling. The slides, rinsed in 2X SSC (pH 7) and air dried, were dehydrated and denatured in 70% formamide (Aldrich) 2X SSC at 73° for 4 min. They were then quickly dehydrated in a graded ethanol series at 0°C and air dried. During this last step, CEP (Chromosome Enumeration Probes, Vysis, IL, USA) -satellite DNA probes for chromosomes X, Y, and 18 directly labeled with different fluorochromes, were used. The probe mix was denatured for 5 min at 73°C in a water bath. Hybridization was carried out at 37°C in a moist chamber for 12 hours. The

slides were then washed with 0.4X SSC-0.3% NP40 for 2 min at 73°C, quickly in 2X SSC-0.1% NP40 at RT and finally mounted with DAPI 125 ng/ml in antifade solution (Vysis, IL, USA). A total of 3500-5000 spermatozoa were analyzed by triple color FISH.

SCORING CRITERIA

The overall hybridization efficiency was >99%. Sperm nuclei were scored according to criteria of Martin et al., 1995. Sperm nuclei are scored only if they are intact, non-overlapping and have a clearly defined border.

In the case of aneuploidy, the presence of sperm tail was confirmed. A sperm was considered disomic if the two fluorescent spots are of same colour, comparable in size, shape and intensity and positioned inside the edge of the sperm head at least one domains apart.

Diploidy was recognized by the presence of two double fluorescent spots, following the above criteria. Observation and scoring was performed on a Leitz Aristoplan Optic Microscope equipped with fluorescence apparatus, with a triple bandpass filter for Aqua, Orange, Green Fluorocromes (Vysis, IL, USA) and a monocrome filter for DAPI.

Patients having the following characteristics are selected:

Diplody represented the 50.24% of total aneuploidy observed before treatment.

-Positive test for the presence of diploidy $\geq$ 0.8% by FISH measurement on ejaculated spermatozoa as described above.

-FSH serum levels < 10 UI/ml

Patients presenting at least one of these characteristics are excluded from the study:

-Body mass index [(BMI = weight (kg)/height (m^2) x 100] <30;

-Presence of infection in the spermatic passageways (i.e., chlamydia, ureaplasma, mycoplasma) at baseline;

-Known autoimmune disorders;

-Thyroid disorders and/or pathologies;

-Chronic hepatopathies;

-Presence of concomitant pathologic conditions that are contraindicated in FSH treatment.

c) Treatment protocol:

Patients are administered with a dose equal to 150 UI/day (2 vials s.c.) on alternate days for 3 months.

d) Aneuploidy/diploidy rates determination by FISH analysis after treatment

The rates of aneuploidy and diploidy in sex chromosomes are measured by FISH analysis after treatment following the protocol detailed above (b).

A reduction in diploidy by -54% was found on a sample size of 8 consecutive patients (95% confidence interval: -20% to -87%; n=3; p=0.02).

A reduction in the frequency of sex chromosome aneuploidy was found on a sample size of 8 consecutive patients.

EXAMPLE 2: Effect of Gonal F on diploidy and aneuploidy in 38 human infertile males.

a) Patient selection:

38 infertile male patients from age 18 to 55 are selected. Their infertility is determined according to the protocol detailed in Example 1.

b) Treatment:

Patients are administered with a dose equal to 150 UI/day (2 vials s.c.) on alternate days for 3 months.

b) End-point measurements:

After 3 months of treatment, the patient undergoes the same examinations that are performed at the start of the study and the FISH test is repeated. The lasting of the effect rate of aneuploidy and especially on diploidy is controlled 90 days after the end of the treatment.

d) Statistical analysis of the measurements:

The statistical significances of differences between averages are calculated using the protocol detailed in Example 1.

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Claims:

1. Use of a substance selected from Follicle Stimulating Hormone (FSH) and FSH variant for the preparation of a pharmaceutical composition for the treatment and/or the reduction of gamete chromosomal alterations in a male.

2. Use according to claim 1 wherein the gamete chromosomal alterations are numerical alterations.

3. Use according to claims 1 or 2 wherein the gamete numerical chromosomal alterations is spermatozoa diploidy.

4. Use according to any of the preceding claims wherein the male is human.

5. Use according to any of the preceding claims wherein the substance is FSH.

6. Use according to any of the preceding claims wherein the substance is rFSH.

7. Use according to any of the preceding claims wherein the substance is administered on alternate days.

8. Use according to any of the preceding claims wherein the substance is administered at or about 75 to 300 IU/mg/dose.

9. Use according to any of the preceding claims wherein the substance is rFSH and the human male is suffering from gamete aneuploidy.

Abstract

The present invention relates to the use of a substance having a FSH activity for reducing gamete chromosomal alterations in a male, more specifically in men suffering from spermatozoa aneuploidy.